KARYOKINESIS DURING CLEAVAGE OF THE ZEBRA FISH BRACHYDANIO RERIO

EDWARD C. ROOSEN-RUNGE

(From the Department of Biology, Brown University)

Introduction

The results presented in this paper have been obtained in the course of a comprehensive study on the periodicity of cell division and mitotic rate during development. A discussion of these results, confined to observations on teleosts, is a necessary preliminary to the more complete investigation, with history and literature, to be presented later.

To obtain a definite picture of the rôle of cell division in development, it is necessary to determine not only the number of mitoses which occur at given times and at given places, but also the duration of a single mitosis and the manner in which it proceeds in different stages of development. In spite of many careful investigations on the rate of mitosis, the duration of mitosis at different periods of development has not been sufficiently determined.

The most successful method in investigating the rôle of cell division in development has been that of Richards (1935) and others who tried to determine mitotic activity by means of a mitotic index or the percentage number of dividing cells. However, it is not possible to tell by this method whether mitoses occur at periodic cycles or are evenly distributed, so that the counting at any time will actually furnish a figure which approximates the average mitotic rate. The present paper deals with the manner and duration of mitosis only during cleavage.

The egg of the zebra fish (*Brachydanio rerio*), recently described as a favorable laboratory subject (Roosen-Runge, 1938), is especially adapted to this study, because of its rapid development, its transparency, and more particularly because the cell nuclei can be easily observed in the living egg. Three lines of investigation will be described in this paper, namely, (1) the morphology of the living and of the fixed nuclei; (2) the duration of divisions and of mitotic phases; and (3) their reaction to temperature changes.

Materials and Methods

Information concerning the propagation and raising of the eggs of the zebra fish may be found in an earlier paper (Roosen-Runge). For

observation of the living egg, a slide with a covering about 1 mm. thick of a mixture of bee's wax and paraffine was used. A hole, the diameter of the egg, was then cut through the layer of wax in order to let the light come through, with a glass ring, 22 mm. wide and 9 mm. high, added to prevent currents from moving the egg. The slide was immersed in water in a large dish of about 150 cc. capacity, to insure an abundant oxygen supply. The egg was then oriented in the hole and all observation carried on, with the slide so immersed, by means of a water-immersion lens (Zeiss, × 40), having sufficient depth of focus to make visible the cells inside the cell membrane. Although the use of an oil-immersion lens is also feasible, it is only useful to check up on details which on the whole can be seen just as clearly with the water-immersion lens.

The temperature was regulated with an ordinary desk lamp shining from varying distances upon the observation dish. This simple device proved sufficient to keep the temperature constant within the range of half a degree Centigrade, since the amount of water in which the egg was kept, being fairly large, made it possible to control the temperature almost continuously during the period of development. Thus the eggs continued to develop under the microscope without the least sign of disturbance from the beginning of the second to the end of the tenth cleavage, that is, for a period of about three hours.

Bouin's solution was used for fixation. The egg membrane and in most cases the yolk were removed after fixation, for it is then quite easy to tear off the membrane from the hardened egg and to remove the brittle yolk. Dioxan or alcohol + benzol was used for dehydration, but the former is the simpler method and, therefore, to be preferred. All sections were stained in Heidenhain's haematoxylin and cut 6 or 8 microns in thickness.

Morphology of the Nuclei

It is impossible to study the nucleus in the living egg before the first cleavage since the delicate structure is then hidden by coarse granules which are whirled up at the base of the cell by the streaming of the protoplasm into the blastodisc. During the first cleavage the streaming still continues, offering some difficulties to the observer. Accurate observation of the nucleus becomes possible only when the cytoplasm clears at the end of the first cleavage. The two nuclei appear as ovals with a longitudinal axis of approximately 18 μ. The outlines are fine and smooth. Two or three, sometimes more, very delicate curved lines divide the nucleus into several sections (Fig. 1). The first signs of mitosis are the swelling of the nucleus and the

appearance of irregularities in its oval shape when tiny indentations can be seen at the poles which appear flattened so that the nucleus assumes a barrel-like shape. Short rays which point toward the center of the nucleus seem to radiate from the depths of the indentations. Very often the nucleus appears to be divided lengthwise into halves by a fine channel which is filled with some substance a shade darker than the nuclear sap.

In the living egg the appearance of the indentations marks the beginning of a very rapid disintegration of the nuclear membrane. The whole circumference appears strongly wrinkled and rapidly fades out, together with the partition lines inside of the nucleus. In a short time no traces of nuclear structures are left. By watching very closely, one can for a moment fancy where the nucleus has been, because this area appears somewhat lighter and free from the tiny granules which are a part of the cytoplasm throughout the cell. Before nuclear structures become visible again, the cell almost completes its division. The changes in the cytoplasm and the shape of the cell during mitosis have already been described (Roosen-Runge, 1938).

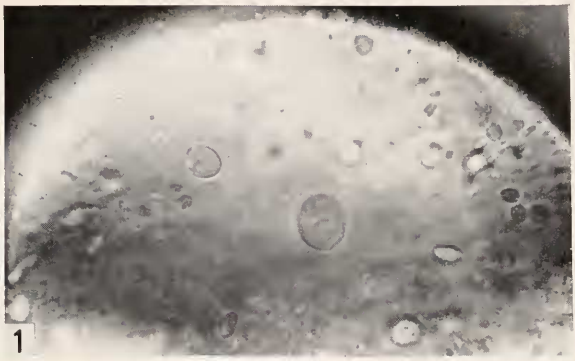
Sometime after the furrow has completely cut through, there appears in the center of each daughter cell a group of tiny dark granules. These granules represent the chromosomes. They swell, become lighter, and finally appear as little circles or vesicles with very distinct outlines. The vesicles go on swelling rapidly until they come into contact with each other, eventually forming one body with a common but irregularly curved contour. The outlines of the individual vesicles remain visible for a time, some of them fading out finally, while others do not disappear until the breakdown of the nuclear membrane in the next prophase.

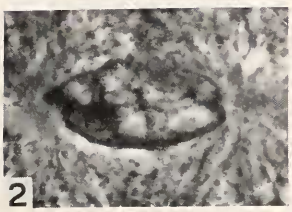
Observations on the living nuclei confirm some of the results obtained from sectioned material. The outstanding feature in the karyokinesis of the teleost blastomeres is the formation of chromosomal vesicles during the telophase. These chromosomal vesicles are quite commonly found in early development and are supposed to persist through the interkinetic phase into the prophase. This interpretation has been made very probable by A. Richards (1917) and B. G. Smith (1929) from the study of sections. It can be proved by the study of living nuclei, in which some of the walls of the vesicles can actually be seen to persist in the interphase nucleus. Some of the walls, however, do not remain visible, but this seems to be due to their thinning out and not to their complete disappearance, since the sections also show some partitions, very dark and distinct, while others are delicate and inconspicuous. In many instances the sectioned nuclei can be seen

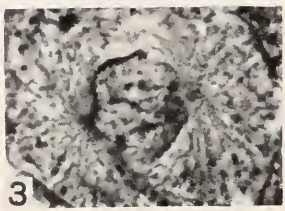
divided into halves, inside of which the vesicles are visible. The halves are separated by a gap, apparently filled with cytoplasm, which corresponds to the observations on the living nuclei. The halves represent the paternal and the maternal parts of the chromosome set, as first described by Moenkhaus (1904) in teleost hybrids, and by many early workers on other forms.

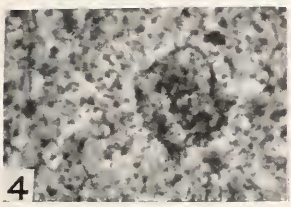
How the vesicles arise from the anaphase chromosomes and how the chromosomes are formed from the vesicles in the prophase, cannot be determined accurately from fixed material, nor do observations of the living nuclei solve any of these problems. Richards (1917) concluded that the vesicles are formed by a swelling of the chromosomes so that finally the walls contain the chromatin material and enclose a space "filled in from the fluid portion of protoplasm." Smith (1929), on the other hand, studied the karyokinesis in *Cryptobranchus* eggs and found that the vesicular membrane was of cytoplasmic origin, developed under the influence of the chromosome within. Pictures like those of Smith certainly cannot be seen in sections of either *Fundulus* or *Brachydanio* eggs. The study of living nuclei only confirms the impression that the chromosomes actually swell during the telophase and that the vesicular wall represents the surface of the chromosome rather than a structure formed *de novo* from the cytoplasm. My own material does not show some of the details as distinctly as they appear (according to Richards) in *Fundulus*, although the formation of the vesicles and their persistence through the interphase could be clearly seen in the sections as well as in the living egg. Nevertheless, the behavior of the chromosome material still remained puzzling. That the reader may be better able to appreciate its actual appearance, I have used photographs (see plate) rather than drawings, as Richards and others have done. Attempts at drawing present possibly too great a temptation to express a prejudiced interpretation not justified by the actual material.

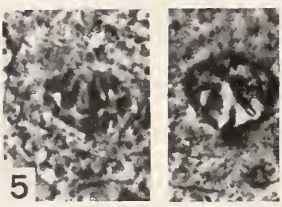
The prophase stage in the karyokinesis of the living blastomere has already been described. The appearance of the nucleus as a whole corresponds very well with the observations of the sections. Because of the rapidity with which the chromosomes reappear and arrange themselves, only a few figures in these phases will be found in material fixed at random. However, by closely watching the living nuclei and taking into account the time necessary for sufficient penetration of the fixing fluid to arrest the mitosis (about half a minute for Bouin's fluid), it is possible to fix material in any desired stage. It can then be seen that the individual chromosomes become clearly visible only immediately before the breakdown of the nuclear membrane. They seem to begin

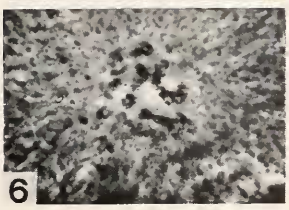












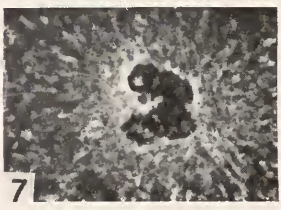


PLATE I

EXPLANATION OF FIGURES

FIG. 1. One of the eight blastomeres of a zebra fish egg, living. Nucleus with a few partition lines within, in the center. × 500.

FIGS. 2–7. Nuclei, fixed in Bouin's, Heidenhain's haematoxylin, 6–8 μ. × 1100. The different sizes of the nuclei are due to their belonging to different cleavage stages.

FIG. 2. Prophase in the beginning. Vesicles still visible. Indentations at the poles.

FIG. 3. Advanced prophase, chromosomes appearing.

FIG. 4. Chromosomes forming metaphase plate. Outline of nucleus still visible.

FIG. 5. Nuclei from cells after the twelfth cleavage, showing typical spireme formation in the prophase.

FIG. 6. Early telophase. Formation of chromosomal vesicles.

FIG. 7. Late telophase. Vesicles in contact. Paternal and maternal half of chromosomes apparently separate.

the arrangement into a plate while still inside of the membrane (Fig. 4). This fact has been confirmed by observations on the living eggs of another teleost, *Epiplaty chaperi*, in which the chromosomes are somewhat more easily discernible in life. Directly after the breakdown of the nuclear membrane the chromosomes can be seen arranged in a metaphase plate but very soon afterwards they begin separating. This observation shows that the disappearance of the membrane actually occurs relatively late. Individual chromosomes inside the separate vesicles, as pictured by Richards, could not be found in sections of the zebra fish egg.

The Duration of Cell Division

The absolute duration of cell division varies tremendously in different animals, and in different cells of the same animal, despite the fact that karyokinesis is supposed to occur essentially in the same way in all of them. The duration of mitosis is characteristic for the different kinds of cells. It can only be measured accurately through the direct observation of living material. The relative time of mitotic phases has been estimated by using the percentage number of cells active in the different stages, but in many cases, as will be pointed out later, this method is very erroneous. It seems, therefore, that direct observation is the safest method for determining the relative intervals in cell division.

The most considerable error in measuring the duration of cell division in life arises from the difficulty in finding any definite point of departure. Neither the beginning of the prophase nor the last stage of the telophase can be defined accurately, so that only a very few events are established sharply enough to serve as marks by which stages may be measured. In the blastomeres of the zebra fish the swelling of the nucleus at the beginning of the prophase, the breakdown of the nucleus, the appearance of the furrow, the completion of the furrow, the first appearance of the chromosomes in the telophase, and finally the completion of the rounded nucleus, furnish seven criteria of very different value. The time of the formation of the furrow, which means the division of the cytoplasm, can only be used indirectly for the determination of karyokinetic stages, although it may serve to subdivide the interval in which nuclear structures cannot be observed at all. The moment when the nucleus seems completely rounded and smoothly outlined is almost impossible to define, and its determination involves a considerable error. The swelling of the nucleus in the early prophase is also difficult to observe, but it is possible to determine its approximate beginning somewhat better with the aid of a micrometer

eye-piece, the scale of which will permit accurate observation of small changes in size. The reappearance of the chromosomes as tiny granules in the telophase is an event more easily determined. Under favorable conditions it is quite possible to watch the optically empty central area of the cell and to see the chromosomes become visible. I estimate the possible error under optimal conditions to be not more than 30 seconds or 3 per cent of the whole time of cleavage. By far the best mark, because of its rapidity of occurrence, is the breakdown of the nucleus. The nuclear membrane not only disappears in from 15 to 30 seconds, but the onset of this event is foreshadowed by a series of preparatory events, namely, the swelling of the nucleus and the wrinkling of the membrane, which makes it possible to predict the time of breakdown quite accurately. The error in determining the precise time of this occurrence is certainly not greater than 15 seconds, which is about 1.5 per cent of the whole time of cleavage. We have thus found two marks which seem reliable, because their errors can be estimated with considerable accuracy at only 1.5 to 3 per cent of the entire duration of cleavage. All other marks certainly have a higher error in determination, and if they are to be used for an estimate of the duration of the mitotic phases, this uncertainty has to be kept in mind.

The time for each cleavage from the first to the tenth is almost the same in different eggs, provided that a constant temperature is maintained and the oxygen supply is sufficient. During the process of cleavage the cell divisions follow each other without a typical resting stage, therefore the cleavage time was measured from the breakdown of the nucleus to the breakdown of the daughter nuclei. In Table I the results are compared with those of Jordan and Eycleshymer (1894) on amphibian blastomeres. The numbers concerning the zebra fish egg are all averages of at least 10 eggs. It can be seen from Table I that in every species the divisions show a characteristic duration. In four of the six animals the divisions show a trend towards acceleration before they finally begin to slow down. (The more complicated curve for the *Amblystoma* egg cannot be discussed here.) The turning point for this trend comes at different times. In the egg of the zebra fish the acceleration is at its height during the fifth cleavage. It seems significant that this is the last division when only one cell layer is involved, for the sixth cleavage is horizontal and divides the blastoderm into two layers. The sixth cleavage takes a slightly longer time than the preceding division, and from then on the process of cleavage gradually becomes slower and slower. Acceleration and retardation seem to involve the whole mitotic process uniformly and not any of its

phases differentially. Only during the ninth and tenth cleavages has a prolonged interkinetic phase been recorded, but as the error in determining this phase is even greater than for any other, no conclusion can be drawn from observations made at these stages of development.

TABLE I

Duration of cleavage divisions in amphibian and teleost eggs.* The times enclosed in brackets refer to individual cases and are not averages.

Temperature, ° C.	*Amblystoma punctatum*	*Rana palustris*	*Diemectylus viridescens*	*Bufo variabilis*	*Epiplaty chaperi*	*Brachydanio rerio*
	18	18	18	18	24	25
Duration of Cleavage Divisions						
Fertilization to first cleavage	10 hrs.?	4–5 hrs.	10 hrs.	4–5 hrs.		25 min.?
First to second cleavage	1 hour 50 min.	1 hour 15 min.	2 hrs.	1 hour 5 min.	2 hrs. 2 min.	20 min.
Second to third cleavage	1 hour 55 min.	1 hour 15 min.	1 hour 45 min.	1 hour	44 min.	19½ min.
Third to fourth cleavage	2 hrs.		1 hour 40 min.	1 hour	(43 min.)	19 min.
Fourth to fifth cleavage	1 hour 40 min.		1 hour 50 min.	1 hour	(41 min.)	18 min.
Fifth to sixth cleavage	(1 hour 35 min.)		(2 hrs. 45 min.)		(39 min.)	17½ min.
Sixth to seventh cleavage	(1 hour 25 min.)		(2 hrs. 45 min.)		(39 min.)	18½ min.
Seventh to eighth cleavage	(1 hour 25 min.)		(2 hrs.)		(40 min.)	19 min.
Eighth to ninth cleavage	(1 hour 25 min.)					20½ min.
Ninth to tenth cleavage						20 min.

* The data on amphibian eggs are taken from Jordan and Eycleshymer (1894).

In measuring the relative duration of the mitotic phases every cleavage can, of course, be observed. Most observations, however, were made during the sixth to ninth cleavages, since these stages had to be studied also for the periodicity of divisions, which will be discussed later. The arbitrary definition of the stages is obviously

a matter of terminology so long as the fundamental mechanism of mitosis is not understood.

The prophase was defined as extending from the first swelling of the nucleus until the break-up of the membrane. The time from the break-up until the chromosomes reappeared was assumed to be the duration of the metaphase plus the anaphase. As to the duration of both of these phases, it can only be stated that the metaphase is much shorter than the anaphase. This is true for two reasons, namely: (1) Nuclei which were observed up to the breakdown of the membrane and then immediately fixed always showed the chromosomes already slightly apart, and (2) the very rapid passing of the metaphase as described can actually be seen in the egg of *Epiplaty chaperi*. From these observations it must be concluded that the metaphase probably takes not much longer than one minute, or about 5.5 per cent of the total division time.

TABLE II

Duration of mitosis and mitotic phases.

Material	Total duration	Prophase	Metaphase	Anaphase	Telophase	Author
	minutes	*per cent*	*per cent*	*per cent*	*per cent*	
Protozoön: *Rhagostoma schüssleri*	32.5	18.5	12.5	18.5	53.5	after Darlington
Protozoön: *Euglypha sp.*	179	22.5	14.0	3.5	60.0	after Darlington
Erythrocytes, *Triton*	150			40.0	46.5	Jolly
The same	180	19.5	12.0	19.5	50.0	Wassermann (after Jolly)
Chorioidea, cartilage in chicken, culture	35	20.0		80.0		Strangeways
Blastomeres, *Brachydanio*	16	18.5		55.5	26.0	
The same, interkinetic phase counted as telophase	18	16.5		50.0	33.5	Roosen-Runge

In Table II, some results on the relative duration of mitotic phases in various animals have been compared. They were all obtained by direct observation. Interesting data like those of Lewis and Lewis

(1917) have been omitted since they are given too inaccurately for the present purpose. They seem, however, not to be in general disagreement with the figures presented here. The significance of the data compared lies in the fact that they agree surprisingly well, in spite of the different kinds of material used by the different investigators as well as the great disparity in the observations made with relation to the total duration of mitosis and the definition of its phases.

The relative time for the prophase varies only from 18.5 to 22.5 per cent in cells as different as those of protozoa, chicken cartilage, and fish blastomeres. The reported times of the metaphase vary also only slightly. However, in both the anaphase and the telophase there is considerable variation although it is smaller in the anaphase than in the telophase.

TABLE III

The duration of cleavage divisions under different temperatures.* The times are minutes.

Cleavage	2	3	4	5	6	7	8	9	10
23° C.						(21)			
23½		(20)	(20)			(21)			
24						20	19.5		
24½	21		19	(20)	(19)				
25		19.5	18.5	18.5	18	18.5	19	19.5	20
25½			(18)	18	17	17	18		
26			(17)				17.5	18	20
26½						(16)			

* The times enclosed in brackets refer to individual cases and are not averages.

The telophase in *Triton* erythrocytes is reported to take 50 per cent of the total time of mitosis, and in protozoa 53.5 and 60 per cent. Lewis and Lewis state that the telophase "which can be more accurately recorded than the other phases, shows a striking similarity in all types of cells and much less variation." If we take their telophase and reconstruction periods together as corresponding to the definition of the telophase used here, we find that the telophase in cultures of chicken mesenchyme and smooth muscles lasts about 50 per cent of the whole time of division, while the telophase of the zebra fish blastomeres takes only about half of this relative time, that is, 26 per cent. Even if the interkinetic phase, the delimitation of which is not at all clear, is added quite arbitrarily to the telophase, there is not more than 33.5 per cent of duration time accounted for. The certainty with which this result is obtained leads to the conclusion that the relative shortness of the telophase is actually significant for the type of karyokinesis we are dealing with, which involves the formation of

chromosomal vesicles in the telophase, and has no actual "resting phase."

The effect of different temperatures on the duration of cleavage is shown in Table III. The results cover only a part of the large range of temperature which the eggs can stand. The only conclusions, therefore, which can be safely drawn are that the duration of cleavage divisions is influenced by even slight changes in temperature, but that the general trend of acceleration for the first six cleavages, and the following retardation, are practically unchanged so that the duration of mitosis may be said to be constant under constant conditions. Many investigators, however, have found the duration of mitosis varying up to several hundred per cent for the same kind of cells. Observations of cells in tissue cultures in particular have yielded results which were very inconsistent with respect to the total duration of division. In all these cases the inconsistency can be attributed only to varying conditions of nutrition, oxygen supply, and temperature. A comparison of the results given in the literature and the observations on the eggs of the zebra fish, show that under constant conditions the duration of mitosis is constant and characteristic for the different types of cells.

Discussion

The process of cleavage is characterized by continuous and often synchronous cell divisions, which frequently follow a definite pattern. In general there is no morphological differentiation during cleavage, but very often there is a segregation of different materials in different cells. At the end of the cleavage period there is a break in the development, the divisions cease to be continuous and synchronous, and the period of cell migration and arrangement begins, often together with the first histological differentiation. On the other hand, cleavage is regarded as "but a continuation . . . of that series of cell-divisions which has been going on uninterruptedly, though with periodic pauses, since the most remote antiquity. The divisions of the egg during cleavage are in all essentials of the same type as those of adult cells; such differences as may appear—e.g., the prominence of asters, the frequent asymmetry of the amphiaster, and the consequent inequality of cleavage—are of minor importance, though often interesting for analyzing the mechanism of mitosis." (E. B. Wilson, 1928, page 981.) The general conception is that cleavage divisions are dynamically somewhat different from the divisions in the older animal, but that their variation is not correlated with any essentially different mechanism. There are, however, observations which point to a difference in mechanism. Investigators of the chromosomal vesicles, which so

frequently occur in the telophase of cleavage divisions, have often suspected that this particular feature of mitosis might be immediately connected with the fact that cleavage divisions go on continuously and almost without interphases.

The study of karyokinesis in zebra fish blastomeres reveals that the formation of chromosomal vesicles is obviously in itself a process of much shorter duration than the common type of telophase and, furthermore, that it represents a condition which permits of an almost immediate start of the next division without a "resting stage" and without a spireme formation in the prophase. No nucleoli are formed in this type of mitosis. All these features are characteristic only for the divisions during cleavage. About the time of the twelfth cleavage an entirely different type of mitosis appears, which shows no chromosomal vesicles in the telophase, but nucleoli and a very distinct spireme in the prophase (Fig. 5). In my material no transitional forms have been observed between these two types, though it is quite possible that a more thorough investigation may reveal such transitions.

Chromosomal vesicles have been found in the eggs of very many species and almost all classes of animals with the possible exception of birds and mammals. (A review of the literature has been given by Richards, 1917.) The suggestion seems obvious that the type of mitosis which is characterized most strikingly by the formation of chromosomal vesicles in the telophase, is due to some aspect of the division mechanism that is peculiar to the cleavage divisions. We have not yet arrived, however, at any definite conclusions concerning the possibly different dynamics involved.

Summary

The nuclei in the blastomeres of *Brachydanio rerio* can be observed easily in life. They are visible in the prophase and telophase as well as in the interkinetic phase. This discovery is used (1) to confirm and consolidate the results obtained from sectioned material; (2) to fix the blastomeres in any desired mitotic phase; and (3) to determine the duration of mitosis and its phases.

The duration of mitosis and its phases under constant conditions, particularly with respect to temperature, is found to be constant for each cleavage. The time from the breakdown of 32 nuclei to the breakdown of 64 nuclei is 18 minutes at 25° C. This places the cleavage divisions of the zebra fish among the most rapid ever observed. The first six cleavages show a trend towards acceleration, the sixth being the most rapid one. From then on the speed of the divisions slows down. This trend is essentially undisturbed by changes in temperature.

The nuclear divisions during cleavage are characterized (1) by the formation of chromosomal vesicles in the telophase (some of these vesicles can frequently be seen in life to persist through the interphase); (2) by a comparatively short duration of this type of telophase; (3) by a very short, if any, true interphase; (4) by the lack of nucleoli; and (5) by the absence of a typical spireme formation in the prophase.

The very short duration of the telophase has been recorded for the first time. The other observations have been found in the cleavage divisions of a majority of the species examined. In the zebra fish egg they continue until about the twelfth cleavage, when the form of mitosis typical for the adult first appears. It is suggested that this type of mitosis is probably associated with the rapid sequence of divisions and is generally characteristic of cleavage mitoses. The most characteristic feature of this type of karyokinesis is the formation of the chromosomal vesicles, but the shortening of the interphase and telophase, and the lack of spireme formation in the prophase are also obvious.

I am indebted to Professor J. W. Wilson, Brown University, for his very valuable advice and to Professor H. E. Walter for his assistance in editing the paper.

LITERATURE CITED

Darlington, C. D., 1937. Recent Advances in Cytology. Philadelphia.

Jolly, J., 1904. Recherches expérimentales sur la division indirecte des globules rouges. *Arch. Anat. Micros.*, **6**: 455.

Jordan, E. O., and A. C. Eycleshymer, 1894. On the cleavage of amphibian ova. *Jour. Morph.*, **9**: 407.

Lewis, W. H., and M. R. Lewis, 1917. The duration of the various phases of mitosis in the mesenchyme cells of tissue cultures. *Anat. Rec.*, **13**: 359.

Moenkhaus, W. J., 1904. The development of the hybrids between Fundulus heteroclitus and Menidia notata with especial reference to the behavior of the maternal and paternal chromatin. *Am. Jour. Anat.*, **3**: 29.

Richards, A., 1917. The history of the chromosomal vesicles in Fundulus and the theory of genetic continuity of chromosomes. *Biol. Bull.*, **32**: 249.

Richards, A., 1935. Analysis of early development of fish embryos by means of the mitotic index. I. The use of the mitotic index. *Am. Jour. Anat.*, **56**: 355.

Roosen-Runge, E. C., 1938. On the early development—bipolar differentiation and cleavage—of the zebra fish, Brachydanio rerio. *Biol. Bull.*, **75**: 119.

Smith, B. G., 1929. The history of the chromosomal vesicles in the segmenting egg of Cryptobranchus allegheniensis. *Jour. Morph.*, **47**: 89.

Strangeways, T. S. P., 1922. Observations on the changes seen in living cells during growth and division. *Proc. Roy. Soc. London, Series B.*, **94**: 137.

Wassermann, F., 1929. Wachstum und Vermehrung der lebendigen Masse. *Handb. der mikrosk. Anat.*, **1**: 2.

Wilson, E. B., 1928. The Cell in Development and Heredity. MacMillan Co., New York.